

TGFbeta1 induces mast cell apoptosis

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Abstract:

Mast cells are potent effectors of the inflammatory response, playing an important role in atopy, bacterial immunity, and animal models of arthritis, multiple sclerosis, and heart disease. Hence controlling mast cell numbers and responsiveness is essential for preventing inflammatory disease. We demonstrate that the cytokine transforming growth factor (TGF) β 1 is a potent inducer of mast cell apoptosis, a finding that was consistent in cultured mouse bone marrow-derived mast cells, peritoneal mast cells, and human mast cells. Cell death appeared to be caused by TGF-mediated repression of interleukin-3 (IL-3) receptor expression and function, leading to mitochondrial damage and activation of an apoptotic cascade acting via p53 and caspases. Although IL-3 receptor expression was reduced within 1 day of TGF β 1 stimulation, apoptosis required at least 3 days to occur. This delay in onset is postulated to allow protective mast cell effector functions, protecting the host from infection while preventing the establishment of chronic inflammation. Our data support the theory that TGF β 1 is an inhibitor of mast cell survival. The widespread expression of TGF β 1 offers this cytokine as an ideal candidate for control of mast cell homeostasis.

Keywords: transforming growth factor | mast cells | TGF β 1 | IL-3

Article:

The transforming growth factor (TGF) β superfamily is a group of structurally related cytokines that includes TGF β 1, TGF β 2, TGF β 3, activins, and bone morphogenetic proteins. TGF β 1, the prototypic family member, is synthesized as a precursor polypeptide and secreted in a latent form by most tissues and cell types [1, 2, 3]. TGF β family members have tissue-specific stimulatory or inhibitory effects on a variety of cellular functions that include differentiation, proliferation, and apoptosis. A number of studies have implicated TGF β proteins in physiologic processes including inflammation, fibrosis, and angiogenesis. Importantly, TGF β proteins have been linked to autoimmune disease, atherosclerosis, fibrotic disease, and cancer in humans [4, 5]. Studies

showing the inhibitory effects of TGF β 1 on immune cell function support the role of TGF β 1 as a suppressor of immunity and inflammation (reviewed by Blobe et al. [5]). Underscoring the importance of TGF-mediated immunosuppression is the observation that TGF β 1-null mice develop severe inflammation, wasting syndrome, and organ failure leading to death by 3 weeks of age [6, 7].

Mast cell activation results in the release of preformed vasoactive amines and de novo synthesized cytokines, chemokines, and prostaglandins that collectively induce a local or systemic inflammatory response [8]. Although mast cells are responsible for immediate hypersensitivity responses, their widespread distribution in the skin and respiratory tract suggests a role as for these cells as a first-line defense against invading pathogens. In fact, mast cells are now known to be critical for resistance to some bacterial infections [9, 10]. Despite this protective role, unregulated mast cell activation can result in deleterious effects. In fact, mast cells are often associated with human allergic disease and are implicated in mouse models of the autoimmune diseases multiple sclerosis and recently rheumatoid arthritis [11, 12]. Thus the mast cell may significantly contribute to immunity by shifting the balance of protective and pathologic responses.

We recently showed that TGF β 1 inhibited mast cell development and dampened expression and function of mast cell immunoglobulin E (IgE) receptors [13, 14]. These studies raised the possibility that TGF β 1 is a paracrine or autocrine inhibitor of mast cell function. This theory is bolstered by the widespread presence of TGF β 1, which circulates in the blood at concentrations exceeding 30 ng/mL [15]. In support of this, TGF β 1 has been shown to inhibit interleukin (IL)-3-dependent proliferation of peritoneal and bone marrow-derived mast cells (BMMCs) [16, 17]. In addition, TGF β 1 inhibited stem cell factor-mediated rescue of BMMCs from apoptosis induced by IL-3 deprivation [18]. To further our understanding of mast cell homeostasis, we directly measured the effects of TGF β 1 on mast cell survival. Our results show that TGF β 1 is a potent inducer of apoptosis in both mouse and human mast cells. These data support the supposition that TGF β 1 can dampen mast cell activity, a finding that has implications for the treatment of allergic and autoimmune disorders.

Materials and Methods

Cytokines and reagents

Murine IL-3, stem cell factor (SCF), and TGF β 1 were purchased from R&D Systems (Minneapolis, MN, USA). Human SCF was the kind gift of Amgen Corp (Thousand Oaks, CA, USA). Mouse IgE, fluorescein isothiocyanate (FITC)-conjugated rat anti-mouse Kit, phycoerythrin (PE)-conjugated anti-mouse IL-3R β , and PE-conjugated anti-human Kit were purchased from BD PharMingen (San Diego, CA, USA). FITC-conjugated rat anti-mouse IgE was purchased from Southern Biotechnology Associates (Birmingham, AL, USA). Stat5 antibodies were purchased from Upstate Biotechnology (Charlottesville, VA, USA). Brefeldin A was purchased from Sigma (St. Louis, MO, USA).

Mast cell cultures

BMMCs were derived from C57BL/6 or BL6x129 mice by culture of bone marrow cells in complete RPMI medium (cRPMI), supplemented with either 25% WEHI-3 cell-conditioned medium or with IL-3 and SCF (30 ng/mL each). After 3 to 4 weeks in culture, >95% of these cells were mast cells, as judged by morphology and surface expression of FcεRI and Kit (data not shown). These cells were used within 3 months of their maturation. Peritoneal cells were harvested from euthanized animals by lavage of the peritoneum using 5 mL of cRPMI injected into the peritoneal cavity. These cells were cultured in cRPMI/IL-3 + SCF. Human skin-derived mast cell populations, obtained after informed consent and institutional approval, were derived as described previously [19, 20].

Cell viability and apoptosis

Cells were assessed for \geq diploid (viable) or $<$ diploid (apoptotic) DNA content by propidium iodide (PI) staining following cell fixation and permeabilization (PI-DNA staining) as described [21]. Briefly, 200 μ L of cells was removed from cultures and centrifuged in a 96-well v-bottom plate for 5 minutes, then washed in phosphate-buffered saline (PBS) and fixed in 150 μ L of PI fixation buffer (70% EtOH/10% fetal calf serum (FCS) in $1\times$ PBS) for 4 hours to 7 days at 4°C. After fixation, cells were washed with PBS and incubated with PI-DNA staining buffer containing 100 μ g/mL RNase A and 50 μ g/mL PI for 2 to 3 hours in the dark at room temperature. Samples were analyzed by flow cytometry to determine the percentage of the population within the subdiploid DNA marker. To measure changes in mitochondrial membrane potential ($\Delta\Psi_m$), cells were incubated with 1 nM Di(OC₆)₃, purchased from Molecular Probes (Eugene, OR, USA) for 30 minutes prior to analysis by flow cytometry. Caspase-3/7 activity was measured by flow cytometry using the caspase-3/7 fluorochrome inhibitor of caspases (FLICA) assay from Immunochemistry Technologies, LLC (Bloomington, MN, USA).

Tissue culture conditions for cell viability and surface receptor expression

Cells were washed and replated at 3×10^5 cells/mL 200 μ L cRPMI/well using 96-well flat-bottom plates. IL-3 was added to 5 ng/mL, followed by the indicated concentrations of TGFβ1. Cultures were incubated for the indicated times. Every 4 days half the media and cytokines were replaced. Mast cell surface antigen expression was assessed by flow cytometry as described previously [22]. The mean fluorescence intensity of IL-3Rβ or Kit staining was used to determine the percent inhibition of expression caused by the addition of TGFβ1.

RNase protection assay

RNase protection assay were performed using the RiboQuant system (BD Pharmingen) as per the manufacturer's instructions. Pixel intensities of individual bands were obtained using a Typhoon phosphorimager (Molecular Dynamics, Sunnyvale, CA, USA). The ratios of the pixel intensity for each band of interest to the sum of the pixel intensities for the housekeeping genes (L32 + GAPDH) in that lane were determined. Calculations of percent change in expression relative to control conditions were determined by comparing these ratios.

Western blot analysis

Tyrosine phosphorylated Stat5, total Stat5, and actin were detected by Western blotting of total cell lysates (approximately 50 μ g/lane) as described [14]. To determine the percent change in expression, band intensity was first measured by densitometry. The ratio of tyrosine phosphorylated Stat5 to total Stat5 was determined, and ratios were compared between lanes to determine percent decrease in the presence of TGF β 1.

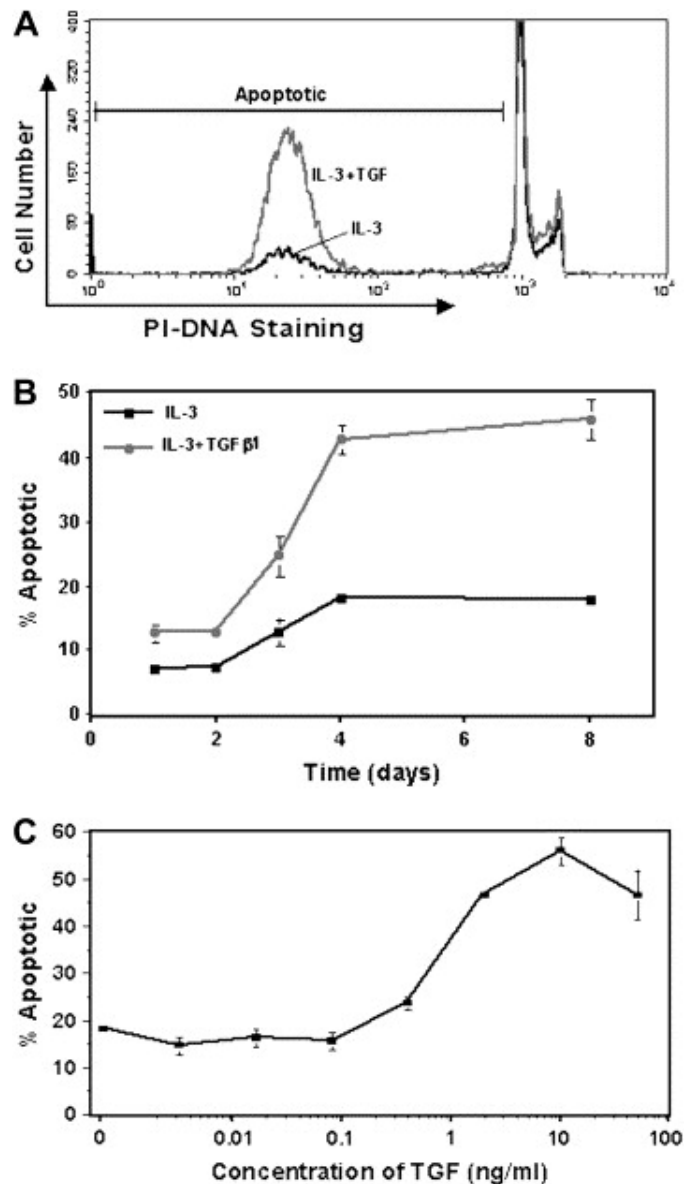


Figure 1. TGF β 1 induces mast cell apoptosis. **(A):** Mouse BMMCs were cultured for 6 days in IL-3 \pm 10 ng/mL TGF β 1. Apoptosis was measured by the presence of subdiploid DNA content after PI-DNA staining, as indicated by marked region in histogram. **(B):** Time course of apoptosis. BMMCs were cultured as in part **(A)** for the indicated days, and apoptosis was measured by PI-DNA staining. Data shown are means and standard errors from at least six samples/point. **(C):** Concentration response for TGF β 1-induced apoptosis. BMMCs were cultured in IL-3 \pm the indicated concentrations of TGF β 1 for 7 days. Apoptosis was measured by PI-DNA staining. Data shown are means and standard errors of six samples/point.

Results

TGF β induces mast cell apoptosis

To determine the effect of TGF on mast cell survival, we cultured mouse BMMCs with IL-3 \pm TGF β 1. BMMCs are primary, IL-3-dependent mast cells that function as a reliable model system for mucosal mast cells [23], hence the effects of TGF on this population are likely to be representative of tissue mast cells. As shown in Figure 1A, BMMCs cultured with TGF β 1 for 6 days exhibited an overt increase in subdiploid DNA content, indicating DNA fragmentation that is consistent with apoptosis. Apoptosis occurred consistently after 3 days of culture, with a peak of 40 to 50% cell death after 4 days of culture (Fig. 1B). This effect was dose-dependent, requiring approximately 2 ng/mL TGF β 1, with maximum apoptosis observed using 10 ng/mL. As stated, this is well within the range of TGF β 1 available in normal human serum [15].

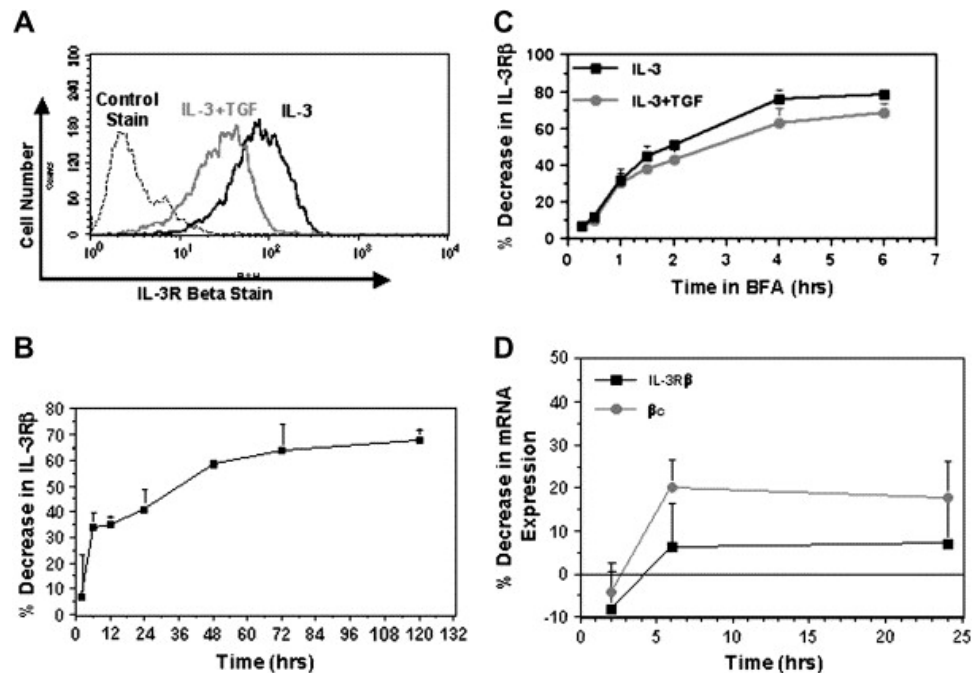


Figure 2. TGF β 1 inhibits mast cell IL-3R β expression. **(A):** Mouse BMMCs were cultured for 3 days with IL-3 \pm TGF β 1 (10 ng/mL). Cells were analyzed for surface IL-3R β expression via flow cytometry. Control stain was performed with PE-coupled IgG. **(B):** Time course of TGF β 1-mediated inhibitory effects on IL-3R β expression. Cells were cultured for the indicated times, and percent decrease in IL-3R β expression was calculated by comparing mean fluorescence intensity of IL-3R β staining from cells cultured \pm TGF β 1, as measured by flow cytometry. Data shown are means and standard deviation of at least three samples/point. **(C):** Effect of TGF β 1 on IL-3R β surface stability. BMMCs were cultured for 24 to 48 hours in IL-3 \pm TGF β 1, after which BFA (20 μ g/mL) was added for the indicated times. The percent decrease in IL-3R β expression was calculated by comparing the change in IL-3R β expression between samples cultured with and without BFA, using flow cytometry. **(D):** Effect of TGF β 1 on IL-3R β mRNA expression. RNase protection assay was used to measure IL-3R β mRNAs from BMMCs cultured for the indicated times in IL-3 \pm TGF β 1. After normalizing to L32 + GAPDH loading controls, percent decrease in mRNA expression was calculated as by phosphorimager as described in Materials and Methods. Data shown are means and standard deviation of three samples/point.

TGF β 1-mediated apoptosis correlates with reduced IL-3 receptor expression

TGF β 1 is known to block expression of the mast cell survival receptor Kit and also inhibits Kit-mediated rescue from factor withdrawal-induced apoptosis [18, 24, 25]. Because IL-3 is the

survival factor employed in these assays, loss of its survival function seemed a plausible means by which TGF β 1 could elicit programmed cell death. To address this possibility, we measured the effects of TGF β 1 on BMMC IL-3 receptor expression by flow cytometry. The IL-3R is composed of an alpha chain paired with either an IL-3-specific β chain or a β c chain shared with IL-5 and granulocyte-macrophage colony-stimulating factor [26]. We found expression of IL-3R α in viable cells to be below the level of detection (data not shown). In contrast, an antibody that recognizes both IL-3R β chains demonstrated robust expression that was significantly inhibited by TGF β 1 (Fig. 2A). Importantly, the reduction in IL-3R β expression preceded the onset of cell death, with 30% inhibition after 8 hours of TGF β 1 stimulation, peaking at 65% inhibition by day 3 of culture, when apoptosis was first detected (Fig. 2B). Because BMDCs die approximately 3 days after IL-3 withdrawal (our unpublished findings), the timing of TGF β 1-mediated inhibition of IL-3R expression fits well with the onset of apoptosis.

We recently demonstrated that TGF β 1 blocked mast cell Fc ϵ RI expression by reducing translational efficiency, with little effect on mRNA expression or protein stability [14]. Like Fc ϵ RI, we found that TGF β 1 had no effect on the rate of surface IL-3R β turnover. The addition of the vesicular transport inhibitor Brefeldin A (BFA) reduced IL-3R β expression at similar rates in cells cultured in IL-3 \pm TGF β 1 (Fig. 2C). Also like Fc ϵ RI, TGF β 1 did not significantly alter IL-3R β mRNA expression, reducing β c message by only 20% at time points preceding or after the onset of IL-3R repression (Fig. 2D). These data support the theory that TGF β 1 inhibits IL-3R expression through posttranscriptional effects that, like Fc ϵ RI, alter translation efficiency. This inhibitory effect occurs with the appropriate timing to explain the onset of mast cell apoptosis.

TGF β 1-mediated IL-3R repression inhibits Stat5 activation and maintenance of mitochondrial membrane potential

If the reduction in IL-3R expression is functionally significant, it should prevent proper activation of the Stat5 pathway, which we have shown to be essential for mast cell survival [27]. To test the effects of TGF β 1 on IL-3-mediated Stat5 signaling, BMDCs were cultured in IL-3 \pm TGF β 1 for 3 days, the point at which IL-3R expression reached its nadir and apoptosis was initiated. After a starvation period to remove any residual IL-3 signaling, these cells were restimulated with IL-3, and Stat5 phosphorylation was measured by Western blotting. As shown in Figure 3A, TGF β 1 reduced Stat5 phosphorylation by 45 to 50%. As we have found that Stat5 expression is necessary for maintaining mitochondrial membrane potential ($\Delta\Psi$ m) [27], we determined the effect of TGF β 1 on $\Delta\Psi$ m via Di(OC₆)₃ staining. As shown in Figure 3B, TGF-stimulated cells exhibited reduced Di(OC₆)₃ staining, shifting toward the spectrum displayed by BMDCs cultured without IL-3, a condition known to induce mitochondrial damage [28]. These results argue that TGF β 1-mediated repression of the IL-3 receptor is biologically relevant, reducing IL-3 signaling to an extent that induces mitochondrial damage.

TGF β 1-induced apoptosis requires p53 expression

Loss of IL-3 signaling has been shown to induce a p53-dependent apoptotic cascade that occurs with mitochondrial damage [29]. The effects of TGF β 1 mirror IL-3 deprivation and hence may employ the p53 pathway for programmed cell death. In fact, we found that p53-deficient (KO) BMDCs exhibited little apoptosis after culture with TGF β 1 (Fig. 4A). This p53 dependency was

confirmed by a substantial reduction in TGF β 1-mediated activation of the effector caspases-3 and -7 (Fig. 4B). These data support the theory that TGF β 1 induces mast cell apoptosis by sufficiently repressing IL-3 receptor expression to mimic IL-3 withdrawal and the p53-dependent/mitochondrial pathway that ensues from this deprivation.

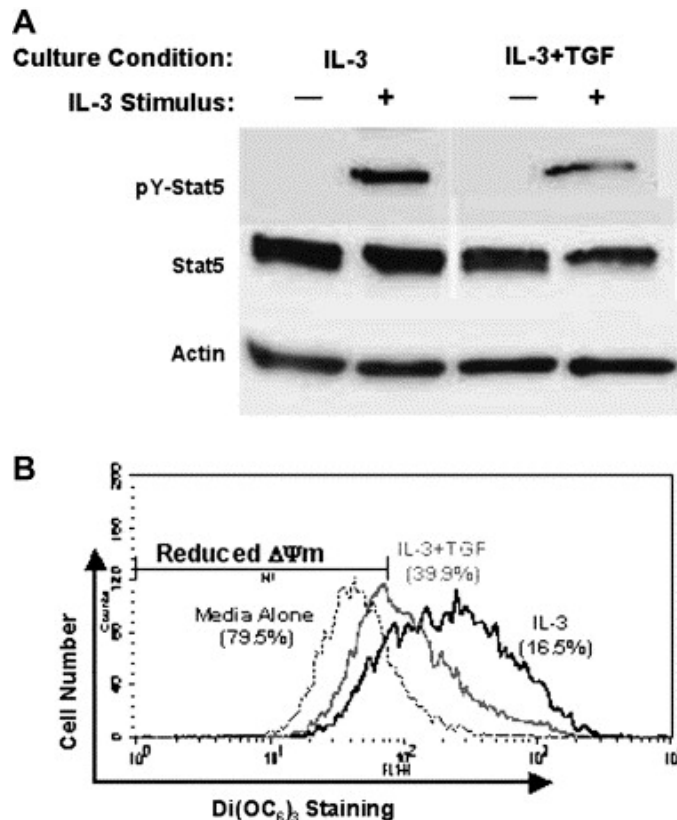


Figure 3. TGF β 1 inhibits IL-3R signaling. **(A):** BMMCs were cultured for 3 days in IL-3 \pm TGF β 1, washed and incubated overnight in the same medium lacking IL-3, then restimulated with IL-3 (100 ng/mL) for 15 minutes. Total cell lysates were subjected to Western blot analysis with phosphotyrosine-specific (pY) anti-Stat5. Membrane was stripped and reprobed with anti-Stat5 and anti-actin. After normalizing to Stat5 expression via densitometry, pY-Stat5 expression was found to be reduced by 44.6% in samples receiving TGF β 1. Similar results were found in two experiments. **(B):** BMMCs were cultured for 4 days in IL-3 \pm TGF β 1 or in media lacking cytokines. Di(OC₆)₃ staining was used to measure changes in $\Delta\Psi$ m, as detected by flow cytometry. Numbers in parentheses indicate the percentage of each population demonstrating reduced $\Delta\Psi$ m.

Factor-independent mastocytoma cells are resistant to TGF β 1-induced apoptosis

If the effects of TGF β 1 are mediated via its blockade of the IL-3 receptor, cells not requiring this growth factor should be resistant to TGF β 1. To test this, we cultured factor-independent P815 mastocytoma cells in IL-3 \pm TGF β 1. These cells possess a mutant, constitutively Kit receptor that drives their continual proliferation. As shown in Figure 5, TGF β 1 stimulation for 6 days had little effect on the viability of P815 cells. We detected no significant change in subdiploid DNA content and no increase in caspase-3/7 activation. Thus these IL-3-independent mast cell tumors are completely resistant to TGF β 1-induced cell death, supporting our hypothesis that TGF β 1 acts through repression of IL-3R expression and signaling.

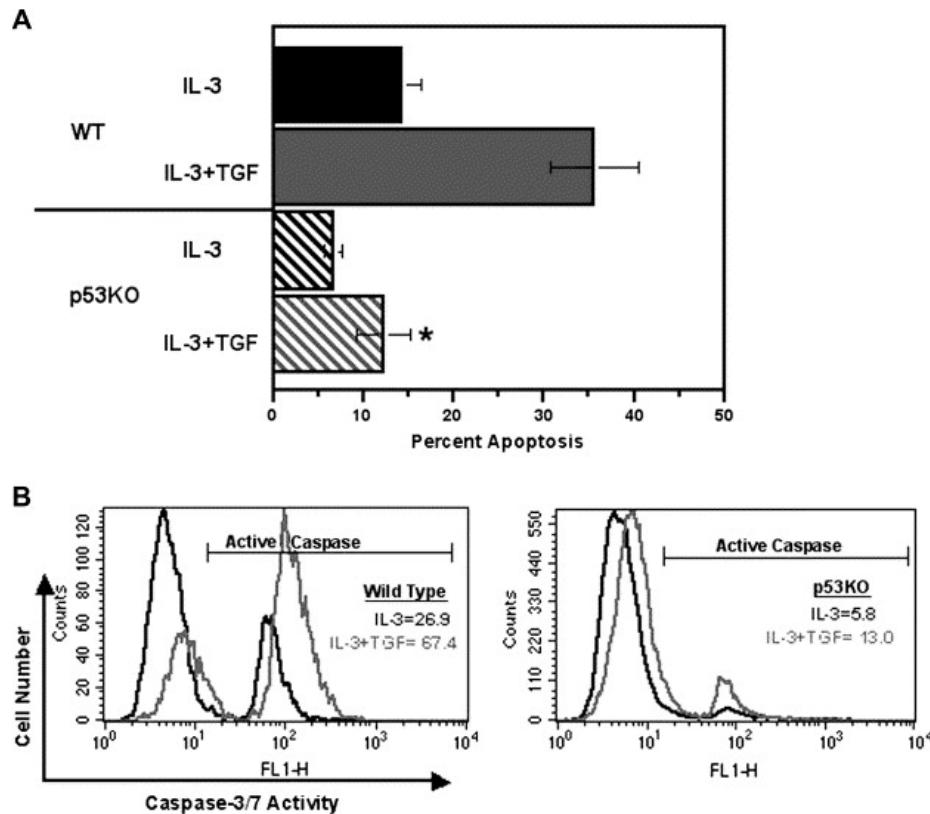


Figure 4. TGF β 1-mediated apoptosis proceeds via the mitochondrion and p53. **(A):** Wild-type (WT) and p53-deficient (KO) BMMCs were cultured in IL-3 \pm TGF β 1 for 6 days, and apoptosis was measured by PI-DNA staining. Data shown are means and standard errors of at least nine samples/point. * $p < 0.001$ by Student's t -test. **(B):** Effect of TGF β 1 on caspase-3/7 activation in WT and p53KO BMMCs. Cells were cultured for 6 days in IL-3 \pm TGF β 1, and caspase activation was measured by flow cytometry as described in Materials and Methods.

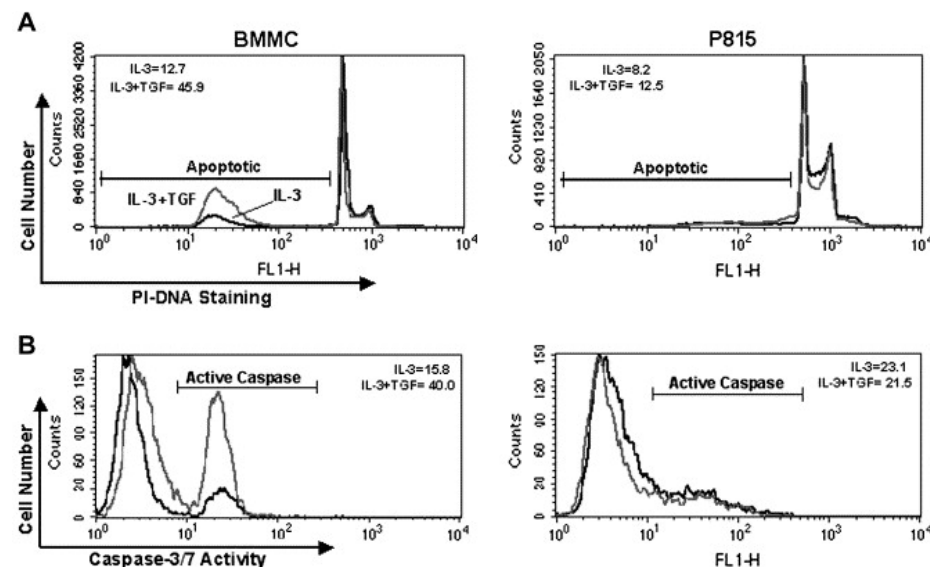


Figure 5. Effect of TGF β 1 on mastocytoma cells. BMMCs and P815 mastocytoma cells were cultured in IL-3 \pm TGF β 1 for 6 days. **(A):** Apoptosis was measured by PI-DNA staining. Percentage of the population demonstrating subdiploid DNA content is indicated. **(B):** Caspase-3/7 activation was measured by flow cytometry using a cleavable substrate, as described in Materials and Methods. Percentage of each population with active caspase is indicated.

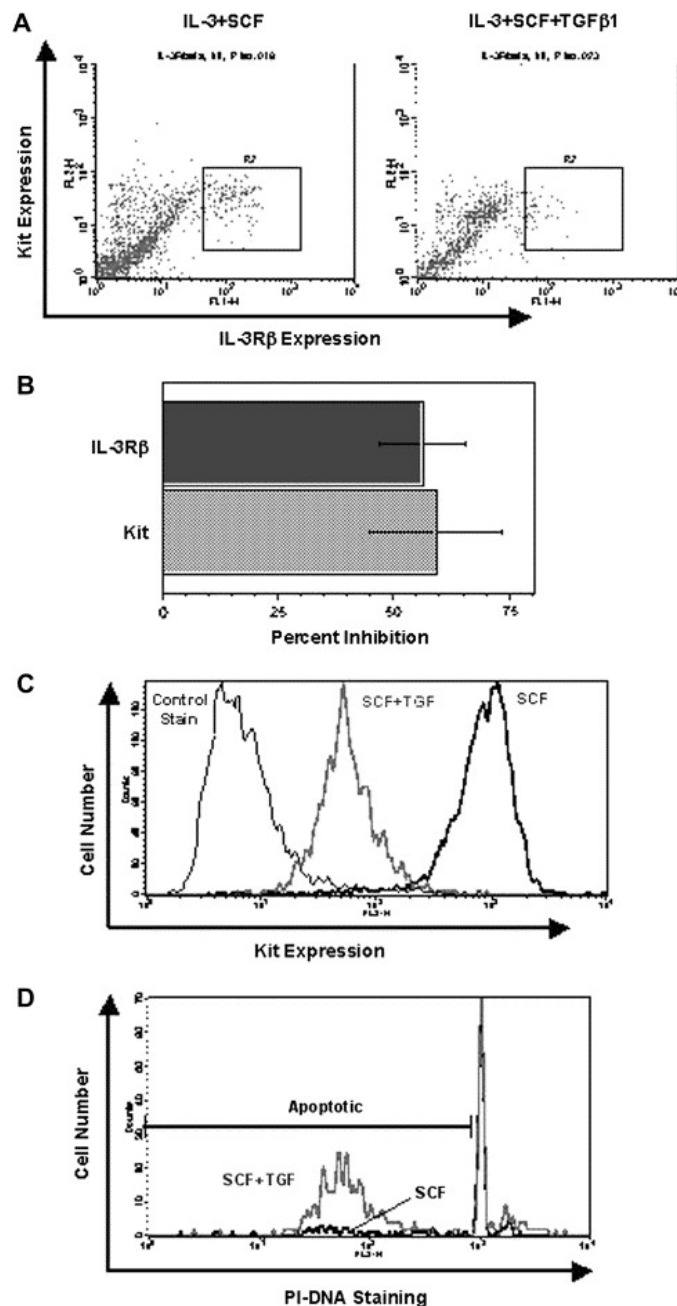


Figure 6. Effect of TGFβ1 on mouse peritoneal and human mast cells. **(A,B):** Mouse peritoneal cells were cultured in IL-3 + SCF ± TGFβ1 for 6 days, followed by flow cytometric analysis with anti-Kit and anti-IL-3Rβ. **(A):** Representative dot plot from 1 of six sample sets. **(B):** Average decrease in Kit and IL-3Rβ expression ± SD. **(C):** Skin-derived human mast cells were cultured in SCF ± TGFβ1. On day 3, surface Kit expression was measured by flow cytometry. **(D):** Apoptosis was measured in cultures described in part (C) on day 7, by PI-DNA staining. Data shown are representative of three independent mast cell cultures that yielded similar results.

TGFβ1 represses Kit expression and induces apoptosis in mouse peritoneal mast cells and cultured human mast cells

Although mouse BMMCs are a reliable model system for mast cell biology, their IL-3 dependency is distinct from the importance of SCF in vivo. To confirm that our findings with

BMMC cultures were consistent in other mast cell populations, we measured the effects of TGF β 1 on freshly isolated mouse peritoneal mast cells and cultured human mast cells, which rely upon SCF for survival and proliferation signals. Unseparated mouse peritoneal cells were used to mimic the effects of TGF in the context of the peritoneal population. These cells were cultured for 6 days in IL-3 + SCF \pm TGF β 1. Mast cells were examined by gating on the Kit⁺ population (Fig. 6A). As shown in Figure 6A and B, TGF β 1 reduced the expression of both IL-3R β and the SCF receptor, Kit, nearly 60%. We also noted that the number of mast cells in these cultures was reduced 80.5% (SD = 14.2, n = 6). Human skin-derived mast cell populations (HSMCs) also confirmed the inhibitory effects of TGF β 1. By day 3 of culture in SCF + TGF β 1, HSMCs showed greatly reduced Kit expression (Fig. 6C). This effect mimicked our observations with the mouse IL-3R. Further, TGF β 1 induced human mast cell apoptosis, as judged by the presence of subdiploid DNA (Fig. 6D). These results were consistent in HSMC derived from three individuals, with apoptosis increasing from 18.2 to 36.1% after the addition of TGF β 1 ($p = 0.02$). Hence the apoptotic effects of TGF β 1 are consistent in murine and human mast cells cultured ex vivo.

Discussion

Mast cell activation is a central facet of atopic diseases such as allergic asthma. The incidence of these diseases has risen dramatically in recent years, emphasizing the importance of understanding and controlling mast cell function. Our efforts have focused on mast cell homeostasis, regulating cell numbers and function. As mast cells provide critical resistance to bacterial and parasitic infections but also elicit inflammation related to atopy, arthritis, multiple sclerosis, and heart disease, this cellular homeostasis may be the fulcrum balancing health and disease.

Mast cells are responsive to many cytokines that can provide homeostatic control. We previously found that the Th2 cytokines IL-4 and IL-10 repress mast cell development, activation, and survival [21, 22, 30, 31, 32, 33]. Although Th2 cells are closely tied to mast cell activity, the presence of TGF β 1 in tissues where mast cells reside and the high level of serum TGF β 1 available during inflammation-induced vasodilation drew our attention to this cytokine. In testing the effects of TGF β 1 on mast cells, we found it capable of suppressing mast cell development and inhibiting IgE receptor expression and function [13, 14]. It was during these experiments that we noted the apoptotic effects of TGF β 1.

We show in the current work that TGF β 1 elicits mast cell apoptosis by repressing IL-3R expression and signaling, resulting in a factor-withdrawal response requiring p53 expression and occurring with mitochondrial damage and caspase activation. This means of inducing apoptosis appears to be lacking in P815 mastocytoma cells. However, these data should be interpreted carefully, as transformed tumor cells can have many defects preventing cell death, including alterations in p53 and other critical apoptotic mediators. As such, it may be that reducing growth factor receptor expression alone on mastocytoma cells would do little to slow their growth. Although our data offer mechanistic insight into TGF-induced cell death, there is still much to understand. It is likely that changes in intracellular signaling pathways by growth factors such as IL-3 and Kit are the prime targets of TGF β 1. By reducing IL-3R and Kit expression, TGF would effectively diminish survival signals. We have previously demonstrated that Stat5 activation is

critical to mast cell survival [27], and indeed this pathway is dampened by TGF β 1. However, this may be one of several signal transduction proteins altered by TGF. A systematic dissection of how TGF β 1 alters IL-3 signaling is needed and is the focus of our current work.

The apoptotic effects of TGF β 1 were consistent in cultured mouse mast cells, peritoneal mouse mast cells, and human mast cells. Moreover, Gebhardt and coworkers recently found that TGF β 1 induced apoptosis in human mast cells derived from intestinal tissue [34]. Thus it is unlikely that our data are related to culture artifacts or species differences. This inhibitory signaling was sensitive, occurring at 1 to 2 ng/mL, which is well below the physiologic concentrations of TGF β 1.

Our studies with TGF demonstrate its role as a potent inhibitor of mast cells, supporting the hypothesis that it can contribute to mast cell homeostasis. This theory is bolstered by the work of several other labs. For example, TGF β 1 diminished IgE-mediated histamine release and tumor necrosis factor alpha production in vitro [35] and inhibited in vivo mast cell responses [36]. It has also been shown to inhibit IL-3-, IL-4-, and SCF-mediated signaling in mast cells, decreasing proliferation or rescue from apoptosis [16, 17, 18]. Lastly, it is interesting to note that polymorphisms in the TGF β 1 promoter segregate with asthmatic families [37]. These data support our interest in TGF β 1 as a mediator of mast cell homeostasis.

The timing of TGF β 1-mediated inhibitory effects is reminiscent of our work with IL-4 and IL-10, which diminished mast cell function and survival after 3 to 6 days of culture [21, 22, 30, 31, 32, 33]. We have postulated that this delay in inhibitory signaling may frame an “inflammatory window” during which mast cell responses elicit inflammation to control infection but after which mast cells are repressed to prevent tissue damage. The effects of TGF β 1 fit well with this theory. For example, TGF β 1 has been reported to elicit mast cell migration [38, 39]. There is also evidence that mast cell proteases activate latent TGF β 1 [40, 41]. Thus it is plausible that TGF acts to draw mast cells to an area of inflammation, where they serve a protective role. Subsequent to prolonged (3-day) stimulation with TGF, in part mediated by latent TGF activation by mast cell proteases, mast cell function and survival would be repressed. This feedback system would restore homeostasis and prevent chronic inflammatory disease.

Our results support the concept that TGF and other inhibitory cytokines normally function in a homeostatic fashion, controlling mast cell activity during an inflammatory response. It is plausible that loss of these control mechanisms through mutation of the cytokines, their receptors, or p53 contributes to inflammatory and autoimmune diseases. Our data emphasize the importance of understanding the molecular mechanisms controlling mast cell homeostasis.

Acknowledgments

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References

1. Massague J. The transforming growth factor-beta family. *Annu Rev Cell Biol.* 1990;6:597–641.
2. Shi Y, Massague J. Mechanisms of TGF- β signaling from cell membrane to the nucleus. *Cell.* 2003;113:685–700.
3. ten Dijke P, Hill CS. New insights into TGF- β SMAD signaling. *Trends Biochem Sci.* 2004;29:265–273.
4. Blobel GA, Schiemann WP, Lodish HF. Role of transforming growth factor β in human disease. *N Engl J Med.* 2000;342:1350–1358.
5. Letterio JJ, Roberts AB. Regulation of immune responses by TGF- β . *Annu Rev Immunol.* 1998;16:137–161.
6. Kulkarni AB, Huh C-G, Becker D, et al. Transforming growth factor β 1 null mutation in mice causes excessive inflammatory response and early death. *Proc Natl Acad Sci U S A.* 1993;90:770–774.
7. Shull MM, Ormsby I, Kier AB, et al. Targeted disruption of the mouse transforming growth factor- β 1 gene results in multifocal inflammatory disease. *Nature.* 1992;359:693–699.
8. Rivera J. Molecular adapters in Fc(ϵ)RI signaling and the allergic response. *Curr Opin Immunol.* 2002;14:688–693.
9. Malaviya R, Ikeda T, Ross EA, Jakschik BA, Abraham SN. Bacteria: mast cell interactions in inflammatory disease. *Am J Ther.* 1995;10:787–792.
10. McLachlan JB, Hart JP, Pizzo SV, et al. Mast cell-derived tumor necrosis factor induces hypertrophy of draining lymph nodes during infection. *Nat Immunol.* 2003;4:1199–1205.
11. Secor VH, Secor WE, Gutekunst C-A, Brown MA. Mast cells are essential for early onset and severe disease in a murine model of multiple sclerosis. *J Exp Med.* 2000;191:813–821.
12. Lee DM, Friend DS, Gurish MF, Benoist C, Mathis D, Brenner MB. Mast cells: a cellular link between autoantibodies and inflammatory arthritis. *Science.* 2002;297:1689–1692.
13. Kashyap M, Bailey DP, Gomez G, Rivera J, Huff TF, Ryan JJ. TGF β 1 inhibits mast cell development. *Exp Hematol.* 2005;33:1281–1291.
14. Gomez G, Ramirez CD, Rivera J, et al. TGF- β 1 inhibits mast cell Fc ϵ RI expression. *J Immunol.* 2005;174:5987–5993.
15. Young DG, Skibinski G, Mason JJ, James K. The influence of age and gender on serum dihydroepiandrosterone sulphate (DHEA-S), IL-6, IL-6 soluble receptor (IL-6sR) and

transforming growth factor beta 1 (TGF- β 1) levels in normal healthy donors. *Clin Exp Immunol*. 1999;117:476–481.

16. Toyota N, Hasimoto Y, Matsuo S, Izuka H. Transforming growth factor β 1 inhibits IL-3- and IL-4-dependent mouse connective tissue-type mast cell proliferation. *Arch Dermatol Res*. 1995;287:198–201.

17. Broide DH, Wasserman SI, Alvaro-Gracia J, Zvaifler NJ, Firestein G. Transforming growth factor- β 1 selectively inhibits IL-3-dependent mast cell proliferation without affecting mast cell function or differentiation. *J Immunol*. 1989;143:1591–1597.

18. Mekori YA, Metcalfe DD. Transforming growth factor- β prevents stem cell factor-mediated rescue of mast cells from apoptosis after IL-3 deprivation. *J Immunol*. 1994;153:2194–2203.

19. Kepley CL. Antigen-induced reduction in mast cell and basophil functional responses due to reduced Syk protein levels. *Int Arch Allergy Immunol*. 2005;138:29–39.

20. Kambe N, Kambe M, Kochan JP, Schwartz LB. Human skin-derived mast cells can proliferate while retaining their characteristic functional and protease phenotypes. *Blood*. 2001;97:2045–2052.

21. Yeatman CF 2nd, Jacobs-Helber SM, Mirmonsef P, et al. Combined stimulation with the T helper cell type 2 cytokines interleukin (IL)-4 and IL-10 induces mouse mast cell apoptosis. *J Exp Med*. 2000;192:1093–1103.

22. Ryan JJ, Desimone S, Klisch G, et al. IL-4 inhibits mouse mast cell Fc3RI expression through a STAT6-dependent mechanism. *J Immunol*. 1998;161:6915–6923.

23. Rottem M, Barbieri S, Kinet JP, Metcalfe DD. Kinetics of the appearance of Fc epsilon RI-bearing cells in interleukin-3-dependent mouse bone marrow cultures: correlation with histamine content and mast cell maturation. *Blood*. 1992;79:972–980.

24. de Vos S, Brach MA, Asano Y, et al. Transforming growth factor-beta 1 interferes with the proliferation-inducing activity of stem cell factor in myelogenous leukemia blasts through functional down-regulation of the c-kit proto-oncogene product. *Cancer Res*. 1993;53:3638–3642.

25. Dubois CM, Ruscetti FW, Stankova J, Keller JR. Transforming growth factor-beta regulates c-kit message stability and cell-surface protein expression in hematopoietic progenitors. *Blood*. 1994;83:3138–3145.

26. Kitamura T, Miyajima A. Functional reconstitution of the human interleukin-3 receptor. *Blood*. 1992;80:84–90.

27. Shelburne CP, McCoy ME, Piekorz R, et al. Stat5 expression is critical for mast cell development and survival. *Blood*. 2003;102:1290–1297.

28. Bojes HK, Feng X, Kehrer JP, Cohen GM. Apoptosis in hematopoietic cells (FL5.12) caused by interleukin-3 withdrawal: relationship to caspase activity and the loss of glutathione. *Cell Death Differ.* 1999;6:61–70.
29. Blandino G, Scardigli R, Rizzo MG, Crescenzi M, Soddu S, Sacchi A. Wild-type p53 modulates apoptosis of normal, IL-3 deprived, hematopoietic cells. *Oncogene.* 1995;10:731–737.
30. Bailey DP, Kashyap M, Mirmonsef P, et al. Interleukin-4 elicits apoptosis of developing mast cells via a Stat6-dependent mitochondrial pathway. *Exp Hematol.* 2004;32:52–59.
31. Gillespie SR, DeMartino RR, Zhu J, et al. Interleukin-10 inhibits Fc epsilon receptor I expression in mouse mast cells. *J Immunol.* 2004;172:31181–31188.
32. Mirmonsef P, Shelburne CP, Yeatman CF, Chong HJ, Ryan JJ. Inhibition of Kit expression by IL-4 and IL-10 in murine mast cells: role of STAT6 and phosphatidylinositol 30-kinase. *J Immunol.* 1999;163:2530–2539.
33. Bouton LA, Ramirez CD, Bailey DP, et al. Co-stimulation with interleukin-4 and interleukin-10 induces mast cell apoptosis and cell cycle arrest: the role of p53 and the mitochondrion. *Exp Hematol.* 2004;32:1137–1145.
34. Gebhardt T, Lorentz A, Detmer F, et al. Growth, phenotype, and function of human intestinal mast cells are tightly regulated by transforming growth factor beta1. *Gut.* 2005;54:928–934.
35. Bissonnette EY, Enciso JA, Befus AD. TGF- β 1 inhibits the release of histamine and tumor necrosis factor- α from mast cells through an autocrine pathway. *Am J Respir Cell Mol Biol.* 1997;16:275–282.
36. Meade R, Askenase PW, Geba GP, Neddermann K, Jacoby RO, Pasternak RD. Transforming growth factor- β 1 inhibits murine immediate and delayed type hypersensitivity. *J Immunol.* 1992;149:521–528.
37. Hobbs K, Negri J, Klennert M, Rosenwasser LJ, Borish L. Interleukin-10 and transforming growth factor-beta promoter polymorphisms in allergies and asthma. *Am J Respir Crit Care Med.* 1998;158:1958–1962.
38. Olsson N, Piek E, Sundstrom M, ten Dijke P, Nilsson G. Transforming growth factor-beta-mediated mast cell migration depends on mitogen-activated protein kinase activity. *Cell Signal.* 2001;13:483–490.
39. Olsson N, Piek E, ten Dijke P, Nilsson G. Human mast cell migration in response to members of the transforming growth factor-beta family. *J Leukoc Biol.* 2000;67:350–356.

40. Taipale J, Lohi J, Saarinen J, Kovanen PT, Keski-Oja J. Human mast cell chymase and leukocyte elastase release latent transforming growth factor-beta 1 from the extracellular matrix of cultured human epithelial and endothelial cells. *J Biol Chem.* 1995;270:4689–4696.
41. Lindstedt KA, Wang Y, Shiota N, et al. Activation of paracrine TGFbeta1 signaling upon stimulation and degranulation of rat serosal mast cells: a novel function for chymase. *FASEB J.* 2001;15:1377–1388.